<myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

I completely understand your concern, having had to review a number of poorly documented PBPK model manuscripts over the years. In the case of the chloroprene model, we were initially just trying to make sure we were correctly reproducing the models used in Himmelstein et al. (2004b) and Yang et al. (2012). Yang et al. provided new in vitro data and the PBPK model was used to illustrate of the predicted species differences in metabolism, but it was not intended to be a risk assessment paper. At this point we are ready to apply the model in a risk assessment and rather than just picking our own preferences we would like to discuss any suggestions you may have regarding changes to parameters where you believe there are better sources.

My understanding is that Allison corrected all the tissue weights and blood flows in our model to agree with Brown et al before we sent it to you, and I'm happy to continue to discuss the best approach for selecting and documenting the values for QPC and QCC. I'll ask Allison to look into your partition coefficient question.

Harvey Clewell
Principal Consultant

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hclewell@ramboll.com

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 14, 2018 3:00 PM
To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

As far as I can tell, Matt H selected cardiac and ventilation parameters to fit his in vivo data for the mouse. Yang et al. (2012) lists the same parameters, did not appear to reconsider whether they are appropriate. The 'documentation' spreadsheet lists the same parameters and cites Himmelstein… who it seems incorrectly cites Brown et al. This is how errors can propagate, which is what we are trying to avoid and address via the QAPP. We have found somewhat frequently that a value in a PBPK paper was incorrectly transcribed from the original source, and sometimes it really does matter.

We are being especially careful here because this is the first time that a human PBPK model might be used without any human in vivo PK data for validation. The process and underlying calculations have to be rock solid.

In fact, if I go to the Andersen et al. (1987) methylene chloride paper (thanks for sending that), it does not list scaled QPC and QCC, but absolute rates, 2.32 L/h for the mouse. Given the BW of 0.0345 kg for the mouse in that paper, I get QPC = QCC = 2.32/(0.0345^0.75) = 29 (28.98 to be more exact).

The difference between 28, 29, and 30 is probably minimal. But for the purpose of the QAPP I need to trace the calculation from the actual source to the value being used, replicate the calculation. If it makes more sense to use the

ventilation rates from the report in the docket, especially for simulating those data, we can go there, but then we'll go ahead and use the exact number (to 2 or 3 figures) we get from there.

We will need to consider what value is appropriate for simulating the bioassay conditions.

I will likely also check Astrand and Rodahl (1970). But, in your 2001 paper QPC = 24.0, QCC = 16.5, which does not match the values in the documentation/current model (27.75 and 12.89). And if I calculate 20 m^3 x 0.67/(24 * 70^0.75), the value I get for QPC is 23.1 (23.07), not 24.0.

Lastly, regarding the PC calculation spreadsheet, the table which lists the values for Himmelstein et al. (2004) has those numbers to like 14 decimal places; i.e., if one selects a value and looks at what's actually in the cell. I would guess that these are calculated values from the underlying original data. Do we have those data? If these are values as sent to you by Matt, that's OK, we'll just want to document that.

Thanks,
-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, August 14, 2018 12:23 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

I agree with your suggestion of changing the value of QCC in the mouse to 28 and citing Andersen et al. (1987). I'll add a discussion to the manuscript about the potential problems associated with using experimental measurements of cardiac output in the mouse for PBPK modeling of exposures. The methods used for estimating resting cardiac output in the studies cited in Brown et al were highly invasive, with a potential to disrupt normal physiology. This problem appears to be greater in mice than in rats, probably due in part to their small size. Regardless, the main point is that the measured resting values represent a basal perfusion rate that is not necessarily informative regarding experimental animals during an exposure. Mel Andersen came up with the idea of estimating liver blood flow (and thus cardiac output) by modeling data on chemicals under flow-limited metabolism conditions but we never published anything about it.

My comment about measuring ventilation was referring to the inhalation study we performed at the Hamner and submitted to the docket in 2010. I presented the results of our modeling of that study at the meeting last month. No parameters were fitted to the data from that study. We used the measured ventilation and assumed a V/Q of 1. The study you quoted in your email below was performed by Matt Himmelstein and published in 2004. I agree with you that the closed chamber data collected by Matt Himmelstein did not provide an adequate validation of the model because he was not able to measure the animals' ventilation rates. That was the impetus for measuring ventilation in the Hamner inhalation study.

Regarding the human, as you increase activity/workload the ventilation rate rises faster that cardiac output so V/Q becomes greater than 1. Fortunately, there is excellent data available from Astrand and Rodahl (1970) on both ventilation and perfusion as a function of workload. When I was running the PBPK model for vinyl chloride for the EPA IRIS assessment I went to the trouble of estimating ventilation and perfusion values at the two standard activity levels used in the risk assessment: EPA default (20 cu.m./day) and OSHA default (10 cu.m./8hrs):

```
PROCED EPA
SET QPC=24,QCC=16.5
END

PROCED OSHA
SET QPC=35,QCC=18
```

END

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Sent: Tuesday, August 14, 2018 8:55 AM
To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

First, if the actual source of this value is other than Brown et al. (1997), then the actual source/citation should be provided.

But this is a direct quote from Himmelstein et al. (p. 30): "The physiological and metabolic parameters obtained from in vitro experimentation were not adjusted except for the alveolar ventilation (QPC) and cardiac output (QCC) as needed to adequately fit the experimental gas uptake data." Then on p. 32:

For both exposure systems, *in vitro* scaling of total CD metabolism was sufficient to explain the *in vivo* gas uptake data. The alveolar ventilation and cardiac output values used for simulation of the experimental gas uptake data were lower than the standard values used for dosimetry modeling (Table 1). The adjustment for the gas uptake simulations gave values for alveolar ventilation that were consistent with those used for modeling of various chemicals (Johanson and Filser, 1992; Medinsky *et al.*, 1994). Plausible explanations proposed by Johanson and Filser (1992) for using approximately 60% of the theoretical alveolar ventilation values reported by Arms and Travis (1988) included reduced ventilation due to sensory irritation, absorption and desorption by the upper airways, or anesthetic effects. For dosimetry modeling, the decision was made to assume the standard ventilation and cardiac parameters based on Brown *et al.* (1997) given the possibility that these parameters were more appropriate for estimating uptake and metabolism associated with bioassay conditions involving repeated whole body exposure.

This is roughly repeated in the discussion. So this contradicts your statements that ventilation was measured – there is nothing in the paper describing such measurements, and if it was there would be no reason to cite Johanson and Filser, Medinsky, since then he would have just used the value he measured. This says pretty clearly that these parameters were adjusted to fit the in vivo PK data (and then switched to more standard values for bioassay simulations).

If Andersen et al. (1987) provides supporting science for using a higher QCC, then that should be cited, so we can go to and check that reference. If there is a significant error in a primary source for physiological parameters (Brown et a.),

then that should have been published at some point. While I know that you and Mel did a lot of this early work, we need peer review citations to meet the requirements of our QAPP.

We can potentially use the value of 28 from Andersen et al. (1987).

All that being said, if it is true that V/Q should be closer to 1, then a value of 2.15 for humans should not be used. I think it would be defensible to apply the same V/Q for humans as needed to fit the mouse data.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Monday, August 13, 2018 4:54 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

The value of QCC for the mouse in the chloroprene model (QCC=30), is similar to the mouse value (QCC=28) in the PBPK model of Andersen et al. (1987) that was used by EPA in the IRIS assessment for methylene chloride, and is consistent with the physiology of ventilation and perfusion.

I was a member of the ILSI RSI committee that resulted in the publication of Brown et al. (1997), and the question of the correct value of QCC to use in a PBPK model for the mouse was a point of discussion at that time. As mentioned in the section beginning on p.453 of Brown et al., while the value of cardiac output used in the PBPK model of Andersen et al. (1987) for the rat is in agreement with the experimental measurements reported in Table 22, the value for the mouse is about double the reported values. The decision to use the higher value of QCC in the mouse was made by Mel Andersen and I when we were at Wright-Patterson AFB, and was the result of comparisons of PBPK models with data for a number of chemicals.

If you convert the alveolar ventilation rates in Table 31 to the same units as the cardiac output in Table 22 (mL/min), the experimental value of 14 mL/min for a 23-30g mouse that is reported in Table 22 of Brown et al. is inconsistent with the experimental value for the ventilation rate in the mouse in Table 31, and would result in a mismatch between ventilation and perfusion (V/Q ratio). Apart from situations involving strenuous activity or disease, ventilation and perfusion rates are maintained at a V/Q ratio close to 1, and a departure from this value by more than 20% is considered of clinical significance. While the data from rats and dogs are consistent with a V/Q ratio close to unity, the mouse data are not.

Species	Alveolar Ventilation (mL/min/100g) (Table 31)	BW (g)	Alveolar Ventilation (mL/min)	Cardiac Output mL/min (table 22)	V/Q ratio
Mouse	116.5	30	35	14	2.50
Rat	52.9	250	132	110	1.20
Dog	23.1	15000	3465	2936	1.18

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 13, 2018 9:41 AM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Jerry, Harvey,
Cc: Also including EPA colleagues, managers

The attached goes on to describe a couple of discrepancies/issues for the physiological parameters, for the most part minor. However, there is a major issue with the QCC for the mouse:

"… for the mouse the QCC and BW yield a total cardiac output of 36 ml/min, while Table 22 of Brown et al. (1997) gives a mean of 14 ml/min, with a range of 12-16 ml/min. Hence the QCC is unrealistically high, should be ~ 11.7 L/h/kg$^{0.75}$. But using QCC=11.7 in the female_mouse_invivo_3.R script results in significant over-prediction of the blood concentration data. This indicates a failure in in-vitro to in-vivo extrapolation, since the increase in QCC effectively increases the rate of metabolism (when flow-limited) to a similar extent. At a minimum, the "parallelogram" approach suggests that a similar correction, a factor of 2.6 times the mean, should be applied for the human QCC when calculating human internal doses."

It's possible that there's a mistake in the in-vitro to in-vivo metabolic extrapolation/calculations that you all can correct. But I flicked through that part of the 'documentation' spreadsheet and see that the calcs are embedded, so I expect all of those check out. What's written above re. a parallelogram option is just my take for possibly dealing with the discrepancy, but we'd need to have an internal discussion about that before determining if it's acceptable.

Also, please provide the full citation for "Clewell et al. (2001)", listed for human physiological parameters. And as indicated before, the spreadsheet refers to another sheet for calculation of the partition coefficients, which wasn't included.

Best regards,
-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 06, 2018 9:30 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; cvanlandingham@ramboll.com; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

I was just getting to that option. See if this will work.

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Managing Consultant

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 9:26 AM
To: Cynthia Van Landingham <cvanlandingham@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Try just changing the file-extension from .zip to .txt and sending as an attachment. I'm trying to unzip the thing from the sharepoint site and just getting a spinning wheel.

From: Cynthia Van Landingham [mailto:cvanlandingham@ramboll.com]
Sent: Monday, August 06, 2018 9:19 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Unfortunately, I believe that the restrictions are on your end not ours. We can all see the files no problem.

Cynthia

Cynthia Van Landingham
Senior Managing Consultant

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 8:18 AM
To: Cynthia Van Landingham <cvanlandingham@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

I tried to just download it. Does it have to be this complicated? We'll be sharing with everyone as part of our open and transparent process…

-Paul

From: Cynthia Van Landingham [mailto:cvanlandingham@ramboll.com]
Sent: Monday, August 06, 2018 9:13 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Paul,

Did you download the zip file to your hard drive and then open or did you open it on the OneDrive site? If you did not try this, selecting all the files and allowing OneDrive to produce one download zip may be best. The chloroprene_model.o_error.txt file is not in the zip we created so may be something that is being created due to the download process. Please read that file to find out if your IT security set-up is preventing files from being extracted.

Thanks, Cynthia

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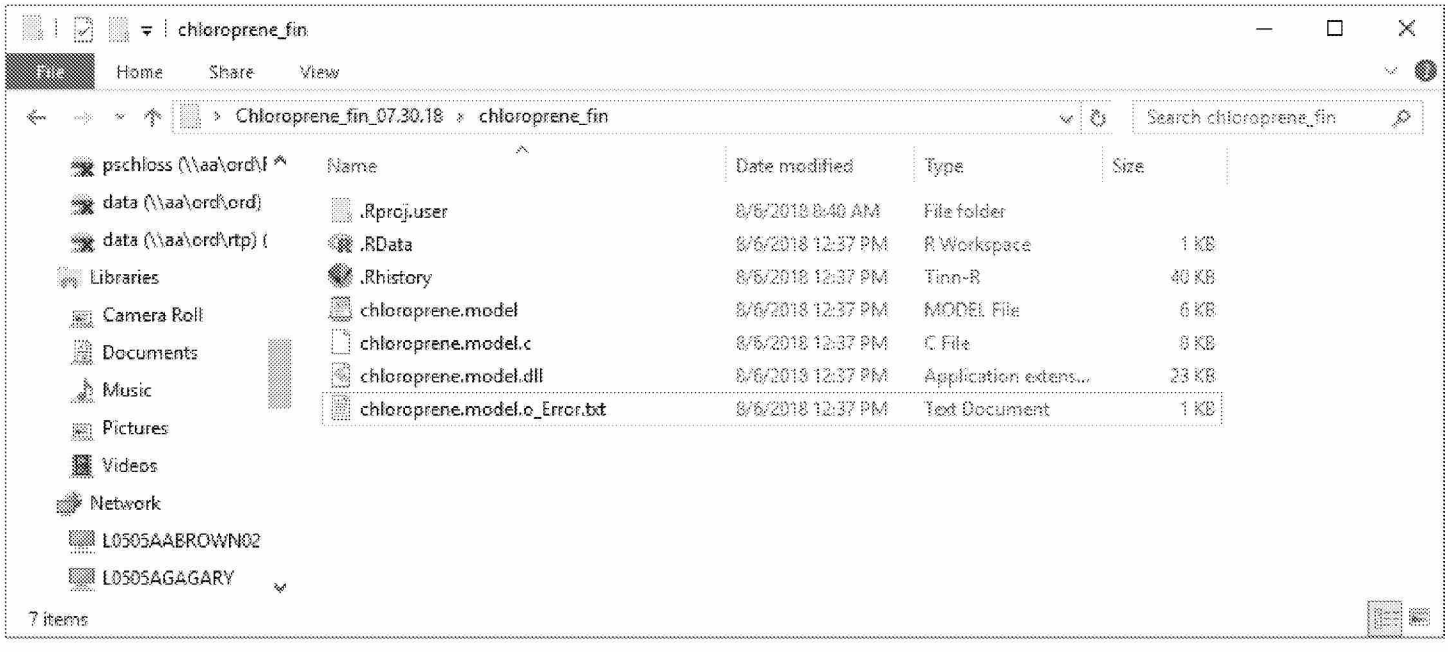
From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 7:53 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

I sent a separate email to Alison. Below is a screenshot of the model folder that I got. There are none of the scripts listed in the Excel 'documentation' file.

Once we have those, give us some time to look at it. Hopefully it's easy enough to figure out, but we can let you and Jerry know if we need a walk-through.

-Paul



From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Friday, August 03, 2018 2:02 PM

To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: transmission of PBPK model for chloroprene

Hi Paul

As promised, we are providing you with the PBPK model for chloroprene written in R, with all the associated scripts and documentation. You should have received a separate email with an invitation to access the files on Microsoft OneDrive. Please let me if you have any problem downloading or opening them. Jerry Campbell would be happy to come over to EPA to help you set up the run environment in R studio and answer any questions you may have about running the model.

I'm looking forward to talking with you about the model and discussing any questions, suggestions, or concerns regarding it. Would it be possible to arrange an initial meeting sometime in the next few weeks. Miyoung Yoon is completing her review of the metabolism parameter scaling approach and I would like to be able to include you in the discussion of her recommendations.

Harvey Clewell

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Message

From: Harvey Clewell [HClewell@ramboll.com]
Sent: 8/22/2018 8:22:00 PM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
CC: Robinan Gentry [rgentry@ramboll.com]; Allison Franzen [AFranzen@ramboll.com]; Miyoung Yoon [myoon@toxstrategies.com]; Sonja Sax [SSax@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Jerry Campbell [JCampbell@ramboll.com]
Subject: RE: transmission of PBPK model for chloroprene
Attachments: chloroprene PC calculations.xlsx

H Paul

With regard to your question regarding the partition coefficient values used in the model, I believe that the tissue:air partition coefficients in the spreadsheet I sent you before were provided to Yuching Yang by Matt Himmelstein, but you'd have to ask him to be sure. They round off to the same values reported in Himmelstein et al. 2004 (Tox Sci 79:28–37) Table 3. The spreadsheet I've attached here uses the rounded off values that were actually published. I prefer using published values rather than raw calculations. Obviously, the rounding makes very little difference in the resulting tissue:blood partition coefficients for the model.

Please let me know if you have any other questions about the model. I was wondering if Jerry and I could get together with you some time to talk about the options for model parameterization to support a risk assessment.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Tuesday, August 14, 2018 4:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <afranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <ssax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

The QA process essentially has two steps:

1) Determine if we can replicate the original study, using those parameters "as is". What's been provided appears successful in this, though I don't see plots for the in vivo gas uptake data of Himmelstein.
2) QA the model code, parameters, data. In this case there is particular attention on the IVIVE. Is it truly predictive?

A component of (2) is tracing all model parameters back to their original source: the paper where the data was first collected/reported, or to a comprehensive physiological review. We have found this is particularly pesky for allometric coefficients, but other derived quantities can also be very hard to replicate. This is why we suggest (and in our own QA do) embedding calculations in a spreadsheet. The first column(s) are numbers exactly as you find them in the source, then the calculations. You've done this for metabolic constants, but not QPC/QCC.

If a parameter is just a little and the results is that there is only a modest fit in the plot of model simulations vs. data (eg, the Himmelstein gas uptake data are fit almost equally well with corrected parameters), that's fine. We then move ahead with the corrected parameters. The assumption is that had the revised plot been submitted for publication, it would have been accepted.

So for now, we aren't looking to refit parameters. But it would help to have the QPC/QCC solidly connected, calculations checked.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, August 14, 2018 3:49 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

I completely understand your concern, having had to review a number of poorly documented PBPK model manuscripts over the years. In the case of the chloroprene model, we were initially just trying to make sure we were correctly reproducing the models used in Himmelstein et al. (2004b) and Yang et al. (2012). Yang et al. provided new in vitro data and the PBPK model was used to illustrate of the predicted species differences in metabolism, but it was not intended to be a risk assessment paper. At this point we are ready to apply the model in a risk assessment and rather than just picking our own preferences we would like to discuss any suggestions you may have regarding changes to parameters where you believe there are better sources.

My understanding is that Allison corrected all the tissue weights and blood flows in our model to agree with Brown et al before we sent it to you, and I'm happy to continue to discuss the best approach for selecting and documenting the values for QPC and QCC. I'll ask Allison to look into your partition coefficient question.

Harvey Clewell
Principal Consultant

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 14, 2018 3:00 PM

To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

As far as I can tell, Matt H selected cardiac and ventilation parameters to fit his in vivo data for the mouse. Yang et al. (2012) lists the same parameters, did not appear to reconsider whether they are appropriate. The 'documentation' spreadsheet lists the same parameters and cites Himmelstein… who it seems incorrectly cites Brown et al. This is how errors can propagate, which is what we are trying to avoid and address via the QAPP. We have found somewhat frequently that a value in a PBPK paper was incorrectly transcribed from the original source, and sometimes it really does matter.

We are being especially careful here because this is the first time that a human PBPK model might be used without any human in vivo PK data for validation. The process and underlying calculations have to be rock solid.

In fact, if I go to the Andersen et al. (1987) methylene chloride paper (thanks for sending that), it does not list scaled QPC and QCC, but absolute rates, 2.32 L/h for the mouse. Given the BW of 0.0345 kg for the mouse in that paper, I get QPC = QCC = 2.32/(0.0345^0.75) = 29 (28.98 to be more exact).

The difference between 28, 29, and 30 is probably minimal. But for the purpose of the QAPP I need to trace the calculation from the actual source to the value being used, replicate the calculation. If it makes more sense to use the ventilation rates from the report in the docket, especially for simulating those data, we can go there, but then we'll go ahead and use the exact number (to 2 or 3 figures) we get from there.

We will need to consider what value is appropriate for simulating the bioassay conditions.

I will likely also check Astrand and Rodahl (1970). But, in your 2001 paper QPC = 24.0, QCC = 16.5, which does not match the values in the documentation/current model (27.75 and 12.89). And if I calculate 20 m^3 x 0.67/(24 * 70^0.75), the value I get for QPC is 23.1 (23.07), not 24.0.

Lastly, regarding the PC calculation spreadsheet, the table which lists the values for Himmelstein et al. (2004) has those numbers to like 14 decimal places; i.e., if one selects a value and looks at what's actually in the cell. I would guess that these are calculated values from the underlying original data. Do we have those data? If these are values as sent to you by Matt, that's OK, we'll just want to document that.

Thanks,
-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, August 14, 2018 12:23 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

I agree with your suggestion of changing the value of QCC in the mouse to 28 and citing Andersen et al. (1987). I'll add a discussion to the manuscript about the potential problems associated with using experimental measurements of cardiac output in the mouse for PBPK modeling of exposures. The methods used for estimating resting cardiac output in the studies cited in Brown et al were highly invasive, with a potential to disrupt normal physiology. This problem appears to be greater in mice than in rats, probably due in part to their small size. Regardless, the main point is that the measured resting values represent a basal perfusion rate that is not necessarily informative regarding experimental animals during an exposure. Mel Andersen came up with the idea of estimating liver blood flow (and thus cardiac output) by modeling data on chemicals under flow-limited metabolism conditions but we never published anything about it.

My comment about measuring ventilation was referring to the inhalation study we performed at the Hamner and submitted to the docket in 2010. I presented the results of our modeling of that study at the meeting last month. No parameters were fitted to the data from that study. We used the measured ventilation and assumed a V/Q of 1. The study you quoted in your email below was performed by Matt Himmelstein and published in 2004. I agree with you that the closed chamber data collected by Matt Himmelstein did not provide an adequate validation of the model because he was not able to measure the animals' ventilation rates. That was the impetus for measuring ventilation in the Hamner inhalation study.

Regarding the human, as you increase activity/workload the ventilation rate rises faster that cardiac output so V/Q becomes greater than 1. Fortunately, there is excellent data available from Astrand and Rodahl (1970) on both ventilation and perfusion as a function of workload. When I was running the PBPK model for vinyl chloride for the EPA IRIS assessment I went to the trouble of estimating ventilation and perfusion values at the two standard activity levels used in the risk assessment: EPA default (20 cu.m./day) and OSHA default (10 cu.m./8hrs):

```
PROCED EPA
SET QPC=24,QCC=16.5
END

PROCED OSHA
SET QPC=35,QCC=18
END
```

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, August 14, 2018 8:55 AM
To: Harvey Clewell <HClewell@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Schlosser, Paul <Schlosser.Paul@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

First, if the actual source of this value is other than Brown et al. (1997), then the actual source/citation should be provided.

But this is a direct quote from Himmelstein et al. (p. 30): "The physiological and metabolic parameters obtained from in vitro experimentation were not adjusted except for the alveolar ventilation (QPC) and cardiac output (QCC) as needed to adequately fit the experimental gas uptake data." Then on p. 32:

For both exposure systems, *in vitro* scaling of total CD metabolism was sufficient to explain the *in vivo* gas uptake data. The alveolar ventilation and cardiac output values used for simulation of the experimental gas uptake data were lower than the standard values used for dosimetry modeling (Table 1). The adjustment for the gas uptake simulations gave values for alveolar ventilation that were consistent with those used for modeling of various chemicals (Johanson and Filser, 1992; Medinsky *et al.*, 1994). Plausible explanations proposed by Johanson and Filser (1992) for using approximately 60% of the theoretical alveolar ventilation values reported by Arms and Travis (1988) included reduced ventilation due to sensory irritation, absorption and desorption by the upper airways, or anesthetic effects. For dosimetry modeling, the decision was made to assume the standard ventilation and cardiac parameters based on Brown *et al.* (1997) given the possibility that these parameters were more appropriate for estimating uptake and metabolism associated with bioassay conditions involving repeated whole body exposure.

This is roughly repeated in the discussion. So this contradicts your statements that ventilation was measured – there is nothing in the paper describing such measurements, and if it was there would be no reason to cite Johanson and Filser, Medinsky, since then he would have just used the value he measured. This says pretty clearly that these parameters were adjusted to fit the in vivo PK data (and then switched to more standard values for bioassay simulations).

If Andersen et al. (1987) provides supporting science for using a higher QCC, then that should be cited, so we can go to and check that reference. If there is a significant error in a primary source for physiological parameters (Brown et a.), then that should have been published at some point. While I know that you and Mel did a lot of this early work, we need peer review citations to meet the requirements of our QAPP.

We can potentially use the value of 28 from Andersen et al. (1987).

All that being said, if it is true that V/Q should be closer to 1, then a value of 2.15 for humans should not be used. I think it would be defensible to apply the same V/Q for humans as needed to fit the mouse data.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Monday, August 13, 2018 4:54 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; cvanlandingham@ramboll.com; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Hi Paul

The value of QCC for the mouse in the chloroprene model (QCC=30), is similar to the mouse value (QCC=28) in the PBPK model of Andersen et al. (1987) that was used by EPA in the IRIS assessment for methylene chloride, and is consistent with the physiology of ventilation and perfusion.

I was a member of the ILSI RSI committee that resulted in the publication of Brown et al. (1997), and the question of the correct value of QCC to use in a PBPK model for the mouse was a point of discussion at that time. As mentioned

in the section beginning on p.453 of Brown et al., while the value of cardiac output used in the PBPK model of Andersen et al. (1987) for the rat is in agreement with the experimental measurements reported in Table 22, the value for the mouse is about double the reported values. The decision to use the higher value of QCC in the mouse was made by Mel Andersen and I when we were at Wright-Patterson AFB, and was the result of comparisons of PBPK models with data for a number of chemicals.

If you convert the alveolar ventilation rates in Table 31 to the same units as the cardiac output in Table 22 (mL/min), the experimental value of 14 mL/min for a 23-30g mouse that is reported in Table 22 of Brown et al. is inconsistent with the experimental value for the ventilation rate in the mouse in Table 31, and would result in a mismatch between ventilation and perfusion (V/Q ratio). Apart from situations involving strenuous activity or disease, ventilation and perfusion rates are maintained at a V/Q ratio close to 1, and a departure from this value by more than 20% is considered of clinical significance. While the data from rats and dogs are consistent with a V/Q ratio close to unity, the mouse data are not.

Species	Alveolar Ventilation (mL/min/100g) (Table 31)	BW (g)	Alveolar Ventilation (mL/min)	Cardiac Output mL/min (table 22)	V/Q ratio
Mouse	116.5	30	35	14	2.50
Rat	52.9	250	132	110	1.20
Dog	23.1	15000	3465	2936	1.18

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 13, 2018 9:41 AM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: transmission of PBPK model for chloroprene

Jerry, Harvey,
Cc: Also including EPA colleagues, managers

The attached goes on to describe a couple of discrepancies/issues for the physiological parameters, for the most part minor. However, there is a major issue with the QCC for the mouse:

"... for the mouse the QCC and BW yield a total cardiac output of 36 ml/min, while Table 22 of Brown et al. (1997) gives a mean of 14 ml/min, with a range of 12-16 ml/min. Hence the QCC is unrealistically high, should be ~ 11.7 L/h/kg$^{0.75}$. But using QCC=11.7 in the female_mouse_invivo_3.R script results in significant over-prediction of the blood concentration data. This indicates a failure in in-vitro to in-vivo extrapolation, since the increase in QCC effectively increases the rate of metabolism (when flow-limited) to a similar extent. At a minimum, the "parallelogram" approach suggests that a similar correction, a factor of 2.6 times the mean, should be applied for the human QCC when calculating human internal doses."

It's possible that there's a mistake in the in-vitro to in-vivo metabolic extrapolation/calculations that you all can correct. But I flicked through that part of the 'documentation' spreadsheet and see that the calcs are embedded, so I expect all of those check out. What's written above re. a parallelogram option is just my take for possibly dealing with the discrepancy, but we'd need to have an internal discussion about that before determining if it's acceptable.

Also, please provide the full citation for "Clewell et al. (2001)", listed for human physiological parameters. And as indicated before, the spreadsheet refers to another sheet for calculation of the partition coefficients, which wasn't included.

Best regards,
-Paul

From: Jerry Campbell [mailto:JCampbell@ramboll.com]
Sent: Monday, August 06, 2018 9:30 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; cvanlandingham@ramboll.com; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

I was just getting to that option. See if this will work.

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Managing Consultant

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 9:26 AM
To: Cynthia Van Landingham <cvanlandingham@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Try just changing the file-extension from .zip to .txt and sending as an attachment. I'm trying to unzip the thing from the sharepoint site and just getting a spinning wheel.

From: Cynthia Van Landingham [mailto:cvanlandingham@ramboll.com]
Sent: Monday, August 06, 2018 9:19 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Unfortunately, I believe that the restrictions are on your end not ours. We can all see the files no problem.

Cynthia

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 8:18 AM
To: Cynthia Van Landingham <cvanlandingham@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

I tried to just download it. Does it have to be this complicated? We'll be sharing with everyone as part of our open and transparent process…

-Paul

From: Cynthia Van Landingham [mailto:cvanlandingham@ramboll.com]
Sent: Monday, August 06, 2018 9:13 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Paul,

Did you download the zip file to your hard drive and then open or did you open it on the OneDrive site? If you did not try this, selecting all the files and allowing OneDrive to produce one download zip may be best. The chloroprene_model.o_error.txt file is not in the zip we created so may be something that is being created due to the download process. Please read that file to find out if your IT security set-up is preventing files from being extracted.

Thanks, Cynthia

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Senior Managing Consultant

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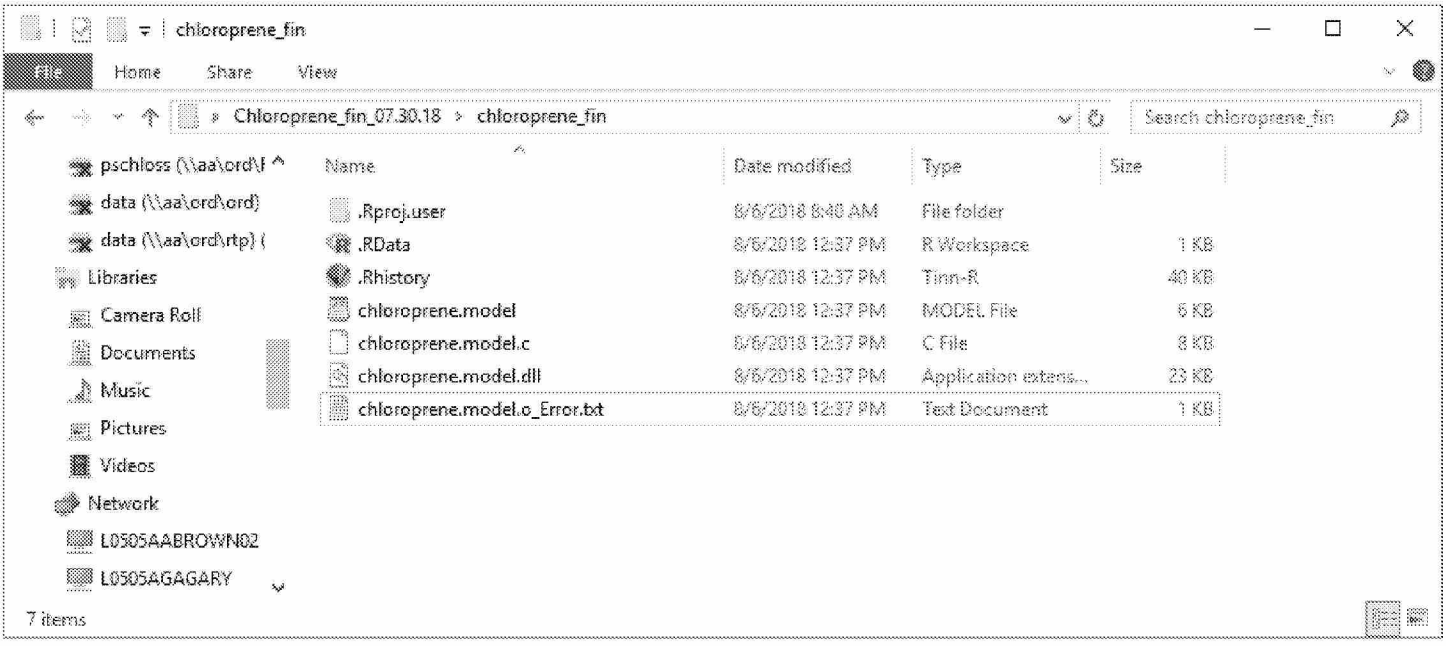
From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Monday, August 06, 2018 7:53 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: RE: transmission of PBPK model for chloroprene

Harvey,

I sent a separate email to Alison. Below is a screenshot of the model folder that I got. There are none of the scripts listed in the Excel 'documentation' file.

Once we have those, give us some time to look at it. Hopefully it's easy enough to figure out, but we can let you and Jerry know if we need a walk-through.

-Paul



From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Friday, August 03, 2018 2:02 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Allison Franzen <AFranzen@ramboll.com>; Jerry Campbell <JCampbell@ramboll.com>; Miyoung Yoon <myoon@toxstrategies.com>; Sonja Sax <SSax@ramboll.com>
Subject: transmission of PBPK model for chloroprene

Hi Paul

As promised, we are providing you with the PBPK model for chloroprene written in R, with all the associated scripts and documentation. You should have received a separate email with an invitation to access the files on Microsoft OneDrive. Please let me if you have any problem downloading or opening them. Jerry Campbell would be happy to come over to EPA to help you set up the run environment in R studio and answer any questions you may have about running the model.

I'm looking forward to talking with you about the model and discussing any questions, suggestions, or concerns regarding it. Would it be possible to arrange an initial meeting sometime in the next few weeks. Miyoung Yoon is completing her review of the metabolism parameter scaling approach and I would like to be able to include you in the discussion of her recommendations.

Harvey Clewell

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Table 1. Tissue: Air Partition Coefficients - Reported in Himmestein et al. 2004; Table 3
CD

Tissue:	B6C3F1 mouse		Fischer rat		Wistar rat		Hamster			Human
Air	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood	7.8	0.1	7.3	0.1	8.0	0.5	9.3	0.3	4.5	0.1
Lung	18.6	5.1	13.5	1.6	11.2	0.5	9.7	0.6	13.3	4.097720509
Liver	9.8	0.9	11.5	0.3	10.9	0.2	10.5	0.5	10.7	1.147726541
Fat	135.3	1.6	124.0	1.5	126.3	1.4	130.1	0.9	128.9	2.73576601
Muscle	4.6	0.8	4.4	0.4	4.0	0.3	5.0	0.2	4.5	0.9
Kidney	13.7	0.6	16.7	0.6	9.4	0.4	8.2	0.3	12.0	0.924177496

Table 2. Tissue:Blood Converted Values from Tisse:air PC
Tissue:Blood

	Mouse	Fischer rat	Wistar rat	Hamster	Human		
Lung	2.38	1.85	1.40	1.04	2.94		PLU
Liver	1.26	1.58	1.36	1.13	2.37		PL
Fat	17.35	16.99	15.79	13.99	28.65		PF
Muscle	0.59	0.60	0.50	0.54	1.00	Slow	PS
Kidney	1.76	2.29	1.18	0.88	2.67	Rapid	PR

Message

From: HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]
Sent: 9/20/2018 11:58:38 AM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]; Jerry Campbell [JCampbell@ramboll.com]
CC: Harvey Clewell [HClewell@ramboll.com]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]
Subject: RE: Chloroprene In Vitro model

Paul,

Glad to see interest in bring the model for chloroprene along. You have caught me in transition. Still same office but now work for Corteva Agrisciences, a Division of DowDuPont. I have access to the data you are asking about but it will take some time for me to comb through it.

Matt

Matthew Himmelstein
DuPont Haskell Global Centers
Phone 302 451 4537

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, September 19, 2018 1:39 PM
To: HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: [EXTERNAL] RE: Chloroprene In Vitro model

Matt, all,

I'm following up to see how things stand regarding the search for additional data. In a separate note Harvey said there should be a report (IISRP?) for the earlier in vitro studies, which it could help to have. Please send any that you have.

As it stands, we have mostly halted our QA review, as it strongly hinges on the equilibration assumption in the in vitro modeling. The code for the in vitro and in vivo models has checked out, issues resolved, and I think all other parameter discrepancies have been resolved – a few changes but none that should make a really large difference.

I realize it might take some time for files to be retrieved from archives and reviewed, but it's now been a couple of weeks since I provided the written details on what we are seeking. Can you tell us where things stand on your end?

The simulations I've run/provided show that the fits to the low concentration in vitro data depend significantly on the assumption that gas-liquid equilibration is not rate limiting, and the data are consistent with the possibility that it is a factor, requiring a fairly large revision in the estimated Km value(s). As is, my conclusion is that there is uncertainty due to the lack of data on the mass transfer rate, and there isn't an easy way that I can think of (or that we are likely to undertake ourselves) for estimating or bounding that uncertainty. The model results are too uncertain to use, given the data and assumptions.

If data are obtained (from archives or newly developed) that show that mass transfer is a factor, it will then be up to Denka/Ramboll to revise the in vitro parameter estimation accordingly, and propagate that into the in vivo model, before we would continue our QA.

As indicated in previous emails, our QA will also involve comparing model predictions to the nose-only in vivo PK data from 2004: the model should be able to fit with parameters adjusted in a way consistent with the hypothesis that there may be an effect of the exposure system on respiration, but this would not be exposure-concentration-dependent. That will require creating model scripts to run these simulations and compare model outputs to the data. While we are prepared to do that work as part of our QA, provided that the mass transfer data become available, we are not planning to begin that work until those data are available and any necessary revisions of the in vitro modeling have been completed. Alternately, Ramboll colleagues could create the scripts in the meantime, which would speed up the QA.

Sincerely,
-Paul

~~~~~~~~~~~~~~~~~~~~~~

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From: Schlosser, Paul
Sent: Wednesday, September 05, 2018 12:06 PM
To: 'HIMMELSTEIN, MATTHEW W' <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt,

Sorry. I was also wondering at the volume being 1.6 mL bigger than advertised, it seemed like a large discrepancy.

A memo is attached, but here is what I've gotten from looking at the code in the appendix of the report you sent:

- Data to indicate that mass transfer resistance is not significant are still lacking.
- The sample volume (VINJ) for all the CP ***oxidation*** experiments in the 2004 paper should be ~ 400 uL, including male mouse and rat liver and lung data. But the code in the report uses 385.8 uL for male data and exactly 200 uL for male data. Is the higher accuracy for the rodent male and human data supported by some measurements?
- Assuming a similar accuracy, the vial volume (VVIAL) for all experiments described in the 2004 paper should be 0.0120 L. This value should be used for male mouse and rat liver and lung data. (We'll use 0.0116 L for the female mouse and rat data and the kidney data.)

Thanks,
-Paul
~~~~~~~~~~~~~~~~~~~~~~

Message

From: Harvey Clewell [HClewell@ramboll.com]
Sent: 1/29/2019 6:51:52 PM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Jerry Campbell [JCampbell@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Sonja Sax [SSax@ramboll.com]
Subject: RE: Chloroprene In Vitro model
Attachments: Denka Chloroprene KG Protocol modified based on EPA comments.docx

Hi Paul, glad you're gainfully employed again.

Now that you're back I wanted to update you on the status of the kg study. Jerry and I made changes to the experimental protocol to address your comments (modified protocol attached), and the laboratory determined that they could perform the revised protocol, but they also discovered that they needed to purchase a shaking water bath. Once that arrives, it will take them at least 2 weeks to conduct the lab work. I'll send you their report as soon as it's complete so we can discuss the results together. Then Jerry can re-estimate the metabolism parameters and incorporate them in the model. Although it is difficult to be precise with the schedule, we are optimistic about completing the PBPK model development in the next several months.

With kind regards
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From: Harvey Clewell
Sent: Thursday, December 13, 2018 4:49 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Sonja Sax <ssax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Hi Paul

Here is the protocol from the laboratory that will be conducting the kg study. Please let me know if you have any comments or suggestions as soon as possible so we can get the study started. Feel free to call me if you want to discuss this.

On another note, have you ever heard back from Matt about finding the original reports on the metabolism studies he performed for his 2004 papers? As I recall, you're concern was to resolve the question of the sample volume for the male lung and liver studies. Were there any other concerns that the reports are needed for?

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Denka Chloroprene Project

12/11/2018

The proposed procedure for the Chloroprene Diffusion Study:

Saline water – 9.0g NaCl in 1L deionized water.
Chloroprene gas standard – 200ppmV (prepared at air lab using neat chloroprene provided by Denka).

12 1040mL (nominal) glass vials (actual volume, which is anticipated to be ~~is~~ 4211-12mL, will be measured) typically used for volatile organic analysis will have 14mL of buffer solution ~~saline water~~ added. They are capped with a Teflon lined septa.

The vials will be placed in a ~~tempering~~ water bath rotating at X rpm, and allowed to equilibrate at 37C. After reaching temperature the pressure in the vials will be adjusted to 1 atmosphere using an open needle placed through the septa.

Two of the vials will be control samples, one the beginning and the other the ending. They will not have chloroprene added. The remaining vials will have 0.52cc of 200ppmV chloroprene added to the headspace of the vial. The contact times will be 5, 10, 20, 30, 45, 60, 120, 180, 240 and 360 seconds. The vials will remain in the tater both during these times. Adding 0.52cc of the 200ppmV chloroprene to 1038cc of headspace should result in a concentration of approximately 10ppmV.

Each vial will have a syringe with a needle long enough to reach below the water inserted. Using another syringe, the 2cc of chloroprene standard will be added to the headspace. The sample will be gently swirled for the time period, then using the syringe placed in the water, 0.53mL will be removed and added to 2mL of deionized water in a separate vial containing a stir bar. This sample will be analyzed for chloroprene using purge and trap and GC\MS.

The control samples will be treated the same, without the addition of the chloroprene.

The total ug of chloroprene absorbed in the saline water can be calculated.

The results of the first run will be provided to Denka for evaluation of the contact times before this process will be repeated ~~5~~ 4 more times.

~~NOTES~~

~~Teklab does not have a GC\MS that is able to have large volumes of gas analyzed on. A sparge vessel with a side arm was attached to a purge & trap sample concentrator. A short piece of small ID tubing was attached with a syringe valve on the end. I was not able to get consistent injections using this method. I was also concerned about the potentially small change in the concentration of the chloroprene with each sample and if that small change could be accurately quantitated. Analyzing the water is the best option available.~~

~~Injecting a very large volume of gas into the headspace of the closed vial can be problematic in that it will pressurize the vial, which will affect the partial pressures of the gases. This can cause the diffusion rate to change causing errors in the test. 2cc into 38cc should not introduce enough pressure to adversely affect the results.~~

Commented [HC1]: If reducing the volumes to work in the smaller vials presents an analytical challenge, the concentration of chloroprene could be increased from 200 ppmV to 800 ppmV.

Commented [HC2]: The vial that was used in the original metabolism experiments was similar to item 854180-U on Sigma Aldrich's website. It is a 10 mL round bottom headspace vial that fits the MPS2 autosampler. The dimensions are 22.5 mm × 46 mm. A flat bottom 10 mL vial is also available that should also be similar to this vial.
Round bottom vial:
[HYPERLINK "https://www.sigmaaldrich.com/catalog/product/supelco/854180u?lang=en&region=US"]
Flat bottom alternative:
[HYPERLINK "https://www.sigmaaldrich.com/catalog/product/supelco/su860029?lang=en&region=US"]

Commented [HC3]: The solution used in the metabolism experiments was 0.1 M phosphate buffer (pH 7.4), MgCl2 (15 mM), EDTA (0.1 mM), glucose-6-phosphate (10 mM), and glucose-6-phosphate dehydrogenase (2 U/mL)

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 9/19/2018 9:31:46 PM
To: Harvey Clewell [HClewell@ramboll.com]; HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]; Jerry Campbell [JCampbell@ramboll.com]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]
Subject: RE: Chloroprene In Vitro model

Sorry, yes, I thought those were nose-only, but those are the data I mean, from the 2nd "Himmelstein et al., 2004" paper.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Wednesday, September 19, 2018 4:41 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Hi Paul

When you talk about the nose-only in vivo PK data from 2004, were you referring to the closed chamber studies that Marina Evans and Elaina Kenyon performed for the Himmelstein et al. 2004b publication?

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Wednesday, September 19, 2018 1:39 PM
To: HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <jcampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt, all,

I'm following up to see how things stand regarding the search for additional data. In a separate note Harvey said there should be a report (IISRP?) for the earlier in vitro studies, which it could help to have. Please send any that you have.

As it stands, we have mostly halted our QA review, as it strongly hinges on the equilibration assumption in the in vitro modeling. The code for the in vitro and in vivo models has checked out, issues resolved, and I think all other parameter discrepancies have been resolved – a few changes but none that should make a really large difference.

I realize it might take some time for files to be retrieved from archives and reviewed, but it's now been a couple of weeks since I provided the written details on what we are seeking. Can you tell us where things stand on your end?

The simulations I've run/provided show that the fits to the low concentration in vitro data depend significantly on the assumption that gas-liquid equilibration is not rate limiting, and the data are consistent with the possibility that it is a factor, requiring a fairly large revision in the estimated Km value(s). As is, my conclusion is that there is uncertainty due to the lack of data on the mass transfer rate, and there isn't an easy way that I can think of (or that we are likely to undertake ourselves) for estimating or bounding that uncertainty. The model results are too uncertain to use, given the data and assumptions.

If data are obtained (from archives or newly developed) that show that mass transfer is a factor, it will then be up to Denka/Ramboll to revise the in vitro parameter estimation accordingly, and propagate that into the in vivo model, before we would continue our QA.

As indicated in previous emails, our QA will also involve comparing model predictions to the nose-only in vivo PK data from 2004: the model should be able to fit with parameters adjusted in a way consistent with the hypothesis that there may be an effect of the exposure system on respiration, but this would not be exposure-concentration-dependent. That will require creating model scripts to run these simulations and compare model outputs to the data. While we are prepared to do that work as part of our QA, provided that the mass transfer data become available, we are not planning to begin that work until those data are available and any necessary revisions of the in vitro modeling have been completed. Alternately, Ramboll colleagues could create the scripts in the meantime, which would speed up the QA.

Sincerely,
-Paul

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From: Schlosser, Paul
Sent: Wednesday, September 05, 2018 12:06 PM
To: 'HIMMELSTEIN, MATTHEW W' <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt,

Sorry. I was also wondering at the volume being 1.6 mL bigger than advertised, it seemed like a large discrepancy.

A memo is attached, but here is what I've gotten from looking at the code in the appendix of the report you sent:

- Data to indicate that mass transfer resistance is not significant are still lacking.

- The sample volume (VINJ) for all the CP ***oxidation*** experiments in the 2004 paper should be ~ 400 uL, including male mouse and rat liver and lung data. But the code in the report uses 385.8 uL for male data and exactly 200 uL for male data. Is the higher accuracy for the rodent male and human data supported by some measurements?

- Assuming a similar accuracy, the vial volume (VVIAL) for all experiments described in the 2004 paper should be 0.0120 L. This value should be used for male mouse and rat liver and lung data. (We'll use 0.0116 L for the female mouse and rat data and the kidney data.)

Thanks,
-Paul

Message

From: Harvey Clewell [HClewell@ramboll.com]
Sent: 5/14/2019 8:40:15 PM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]
Subject: chloroprene
Attachments: Chloroprene PBPK Model Manuscript.docx; Supp Mat B - Supplemental Tables.docx; Supp Mat C - Re-estimation of Metabolism Parameters.docx; Supp Mat E - IVIVE Literature Review.docx; Supp Mat F - Metabolism Parameter Calculations.xlsx; Supp Mat G - PBPK Model Code.docx

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
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Incorporation of *in vitro* metabolism data and physiologically based pharmacokinetic modeling in a risk assessment for chloroprene

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Abstract

A physiologically based pharmacokinetic (PBPK) model for chloroprene in the mouse, rat and human has been developed that relies solely on *in vitro* studies for the estimation of model parameters describing tissue metabolism and partitioning. The predictions of the PBPK model are consistent with *in vivo* pharmacokinetic data from a 6-hr, nose-only chloroprene inhalation study conducted with female B6C3F1 mice, the most sensitive species/gender for lung tumors in the 2-year bioassays conducted with chloroprene. This PBPK model has been applied in a cancer risk assessment for chloroprene using *in vitro* data on the metabolism of chloroprene to reactive epoxides in the lung target tissue of mice and humans. The Inhalation Unit Risk (IUR) estimate obtained with the PBPK model is 178-fold lower than the IUR calculated by the Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) based on inhaled concentration. The lower risk estimate is due primarily to the impact of species differences in lung metabolism. Analysis of model sensitivity indicates that *in vivo* validation studies actually provide little basis for determining whether the model is fit-for-purpose for its application in a risk assessment for chloroprene.

Key Words: chloroprene, inhalation, PBPK, cancer risk assessment

Introduction

Chloroprene (CAS # 126-99-8) is a highly volatile chlorinated analog of 1,3-butadiene that is used in the manufacture of polychloroprene rubber (Neoprene). A cancer risk assessment for chloroprene conducted by the USEPA (2010) calculated an inhalation unit risk (IUR) of $5x10^{-4}$ per $\mu g/m^3$ based on tumor incidence data from female mice exposed to chloroprene for 2 years (NTP 1998; Melnick et al. 1999). The USEPA (2010) assessment used a default cross-species extrapolation approach based on chloroprene exposure concentration, despite strong evidence of quantitative differences in chloroprene metabolism in mice and humans that would have a significant impact on the calculated risk (Himmelstein et al. 2004a,b). The metabolism of chloroprene results in the formation of reactive epoxides that are considered to be responsible for its carcinogenicity in rodents (USEPA 2010).

To determine the potential impact of species-specific differences in the production of these epoxides, a physiologically based pharmacokinetic (PBPK) model was developed in a collaborative research effort between DuPont Haskell Laboratory and the USEPA National Health and Environmental Effects Research Laboratory (NHEERL). *In vitro* measurements of partition coefficients and metabolism parameters for chloroprene in mice, rats, hamsters and humans (Himmelstein et al. 2004a) were used in the PBPK model (Himmelstein et al. 2004b) to predict species-specific dose metrics for the production of epoxides in the lung, the most sensitive tissue in the mouse bioassay. The dose metric chosen for this comparison is consistent with the dose metrics used in previous PBPK-based risk assessments for methylene chloride and butadiene, which are also metabolized to reactive metabolites that are considered to be responsible for the observed carcinogenicity in rodents. Closed-chamber exposures of mice, rats and hamsters were used to validate the PBPK model's ability to predict the pharmacokinetic behavior of chloroprene *in vivo*. The USEPA (2010), however, did not make use of the PBPK model from Himmelstein et al. (2004b) in their risk assessment, citing the lack of blood or tissue time course concentration data for model validation. In addition, USEPA indicated that they did not consider the comparisons of model predictions with the closed-chamber studies to be adequate because the data were limited to chloroprene vapor uptake from the closed chambers.

After the time of the USEPA (2010) evaluation, Yang et al. (2012) provided additional data for refining the PBPK model of Himmelstein et al. (2004b). To supplement the data in Himmelstein et al.(2004a) on liver and lung metabolism in male mouse, male rat, and pooled human cells, Yang et al. (2012) measured liver and lung metabolism in female mouse and female rat, as well as kidney metabolism in male and female mouse, male and female rat, and pooled human cells. The totality of the data from the Himmelstein et al. (2004a) and Yang et al. (2012) *in vitro* metabolism studies was then used to refine the metabolism parameter estimates for the chloroprene PBPK model using Markov-chain Monte Carlo (MCMC) analysis. A comparison of lung dose metric estimates in mouse, rat and human was then performed using the updated metabolism parameters (Yang et al. 2012). These dose metrics were subsequently used in a study comparing genomic responses to chloroprene in the mouse and rat lung (Thomas et al. 2013) and a study comparing human risk estimates derived from mouse bioassay and human epidemiological data (Allen et al. 2014), but to date no *in vivo* blood or tissue time course concentration data have been published with which to evaluate the ability of the chloroprene PBPK model to predict *in vivo* kinetics.

The objectives of the present study were to: 1) characterize the *in vivo* pharmacokinetics of chloroprene via analysis of arterial whole blood concentrations in female B6C3F1 mice during and following a single 6-hour nose-only inhalation exposure, and 2) determine respiratory parameters (breathing frequency and tidal volume) during chloroprene exposure. In this paper we also demonstrate the ability of the refined chloroprene PBPK model to reproduce new *in vivo* validation data and use the PBPK model in an inhalation cancer risk assessment that properly considers species differences in pharmacokinetics and metabolism.

Materials and Methods

Nose-only Exposure Study

Test Substance and Atmosphere Generation

The test substance, β-Chloroprene (CAS # 126-99-8) containing polymerization inhibitors, was supplied by the sponsor as a clear liquid. Exposure atmospheres were generated by metering saturated chloroprene vapor from a stainless-steel pressure vessel reservoir (McMaster Carr,

Atlanta, GA) into the nose-only exposure chamber air supply. The concentrated chloroprene vapor was metered through a mass flow controller (MKS Instruments Inc., Andover, MA) and mixed with HEPA-filtered air approximately six feet upstream of the nose-only inlet. Chloroprene vapor was introduced counter-current to the dilution air to facilitate mixing of the vapors with the dilution air. Chloroprene concentrations were monitored on-line using a gas chromatography system with flame ionization detector (GC-FID). Calibration of the GC-FID for chloroprene analysis was conducted through the analysis of a series of calibration standards produced by introducing pure chloroprene into Tedlar® bags containing known volumes of nitrogen gas (nitrogen was metered into the bag using a calibrated flow meter).

Test Animals and Housing

Female B6C3F1 were purchased from Charles Rivers Laboratories, Inc (Raleigh, NC) at 8 weeks of age and acclimated to their surroundings for approximately two weeks prior to use. Following acclimation animals were assigned to a dosing group by randomization of body weights using Provantis NT 2000, assigned unique identification numbers, cage cards, and housed (1/cage) in polycarbonate cages with standard cellulose bedding. Animals were housed in a humidity and temperature controlled, HEPA-filtered, mass air-displacement room provided by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited animal facility at The Hamner Institutes. This room was maintained on a 12 hour light-dark cycle at approximately 64oC-79oF with a relative humidity of approximately 30-70% (Room monitoring data available upon request). Rodent diet NIH-07 (Zeigler Brothers, Gardners, PA) and reverse osmosis water was provided ad libitum except during exposures. Food and water were withheld from all animals during the chloroprene exposures. Prior to the start of the chloroprene exposure, animals were weighed and their weights were recorded.

The Hamner Institutes for Health Sciences was fully accredited by the AAALAC during the time the study was performed. Currently acceptable practices of good animal husbandry were followed per National Research Council's Guide for the Care and Use of Laboratory Animals (NRC 1996) and were in compliance with all appropriate parts of the Animal Welfare Act (1966). In addition, the study design and protocol were approved by The Hamner Institutes' Institutional Animal Care and Use Committee (IACUC) prior to the initiation of the study.

Inhalation Exposures

Inhalation exposures were conducted at 13, 32, and 90 ppm for 6 hours. Arterial blood was collected at a total of 6 time-points, 0.5, 3, and 6 hours during exposure and 5, 10, and 15 minutes post-exposure. To support collection of whole blood during the exposures, nose only towers were fitted with specially designed nose only exposure tubes. These exposure tubes were manufactured from 50 mL polypropylene bulb irrigation syringes (Sherwood Medical, St. Louis, MO). Three elongated holes (0.625" x 1.125") were drilled into the wall of the syringe to allow access to the thorax of the mouse during chloroprene exposure. A second irrigation syringe was cut to form a sleeve around the first syringe to provide an air tight barrier during the exposures. This sleeve was pulled back during the exposure to allow for the injection of pentobarbital (100 mg/kg) while the animal continued to inhale chloroprene. Arterial blood was removed directly from the mouse via cardiac puncture while the mouse was still housed in the syringe and breathing chloroprene.

Plethysmography

A total of 16 mice (4 per exposure group including air controls) were used for the purpose of collecting tidal volume and breathing frequency. Data were acquired using modified nose-only Buxco plethysmograph tubes for pulmonary function monitoring. Data from control mice were collected prior to the first chloroprene exposure. Plethysmography data from both control and exposed mice were collected for 2-3 hours.

Blood Sampling

Collection of whole blood from chloroprene exposed mice was conducted using nose only towers and specially designed nose only exposure tubes. Whole blood was collected at 0.5, 3, and 6 hours during exposure and 5, 10, and 15 minutes post-exposure. Whole blood collection during chloroprene exposures (0.5, 3, and 6 hour time points) were done using the specially designed nose only exposure tubes mentioned in sections 3.2.1 and 3.2.2 above.

Blood Analysis

Quantification of chloroprene in whole blood was conducted by headspace sampling with analysis by gas chromatography mass spectrometry (GC/MS). The sampling method to be used, headspace analysis, as well as the GC/MS method were based on the previously published